

CD154 LIGAND, AND RECOMBINANT CELLS EXPRESSING IT

The invention herein described relates to cells and/or tissues and/or organs which do not naturally express the cell surface receptor CD40 ligand, CD154, for use, particularly but not exclusively, in therapeutic and cosmetic tissue engineering and/or organ transplantation; compositions comprising said cells and/or tissues; organs comprising said cells/tissues; and methods of therapy and/or cosmetic surgery using said cells and/or tissues and/or organs.

10 Tissue engineering is an emerging science which has implications with respect to many areas of clinical and cosmetic surgery. More particularly, tissue engineering relates to the replacement and/or restoration and/or repair of damaged and/or diseased tissues to return the tissue and/or organ to a functional state. For example, and not by way of limitation, tissue engineering is useful in the provision of skin
15 grafts to repair wounds occurring as a consequence of: contusions, or burns, or failure of tissue to heal due to venous or diabetic ulcers. Further, tissue engineering is also practised during: replacement of joints through degenerative diseases such as arthritis; replacement of coronary arteries due to damage as a consequence of various environmental causes (e.g. smoking, diet) and/or congenital heart disease including
20 replacement of arterial/heart valve; repair of gastric ulcers; replacement bone tissue resulting from diseases such as osteoporosis; replacement muscle and nerves as a consequence of neuromuscular disease or damage through injury.

In addition, organ transplantation has for many years been an established surgical
25 technique to replace damaged and/or diseased organs. The replacement of heart, lung, kidney, liver, bone marrow, and double organ transplantation of, for example and not by way of limitation, heart and lung, are relatively common procedures.

However, in both tissue engineering and organ transplantation a major obstacle to the
30 successful establishment of a tissue graft or organ transplantation is the host's rejection of the donated tissue or organ.

With respect to tissue engineering and organ transplantation, surgeons currently have three types of graft/organ:

- 5 i. an autograft in which a piece of tissue is removed from one area of a patient's body and placed in another location;
- ii. an allograft, in which a section of tissue from one human, for example a cadaver, is grafted onto another human; and
- 10 iii. a xenograft, where tissue is harvested from another species, for example a pig, and placed over the wound area.

Autografts can be problematic due to the availability of suitable tissue and the added trauma to the patient following the removal of the tissue from another part of the
15 body to the wound area. Allografts can be problematic due to the immunological reactivity of the host and/or the availability of donor tissue/organ. Xenografts are even more problematic due to the severe immunological reactivity of the host and the psychological problems relating to the implantation or grafting of tissue/organ from a non- human species onto or into a human body.

20

The body has developed many defences against invasion of foreign organisms. These humoral and cellular defence mechanisms are also directed against foreign antigens expressed by various tissues/organs used in tissue engineering and/or organ transplantation.

25

A general term to cover a number of distinct cell types intimately involved in both a humoral and cellular defence mechanism is white blood cells. Each white blood cell type has a separate role to play in a hosts immune system. Monocytes are large white blood cells that differentiate into macrophages. The macrophages are found
30 throughout the body in various types. For example specialised macrophages include alveolar macrophages in the lungs, mesangial phagocytes in the kidneys, microglial cells in the brain, and Kupffer cells in the liver. Macrophages have many roles and

these include, by example and not by way of limitation, ingestion of infectious agents, antigen presentation to T-lymphocytes and the secretion of agents involved in regulating the immune system (i.e. interleukin-1, complement proteins).

- 5 A pivotal cell-cell interaction between the many cell types of the immune system is between T- lymphocytes (T- cells) and B- lymphocytes (B- cells). T – cells recognise polypeptide antigens presented as peptides via self molecules referred to as the major histocompatibility complex (MHC) on antigen presenting cells such as macrophages. T-cells are divided into cytotoxic T- cells (CTL's) and T- helper cells.
- 10 The latter class of T-cell are able to stimulate B- cell proliferation and mediate immunoglobulin isotype switching to produce antibody isotypes (IgG, IgA, IgD, IgM, IgE) to specific peptide antigens.

- The regulation of biochemical and physiological responses to foreign antigens is, by
- 15 and large, mediated through intercellular and/or intracellular receptor mediated activation via ligand binding. Typically, ligands which interact with receptors to bring about a suitable biochemical/metabolic response are known as agonists and those that prevent, or hinder, a biochemical/metabolic response are known as antagonists.

20

- The interaction of T helper cells and B-cells involves receptor/ligand binding. The B-cell CD40 receptor (CD40 is a monoclonal antibody which recognises the receptor) and the T-cell ligand gp39, referred to hereinafter as CD154 (CD 154 is a monoclonal antibody which recognises gp39) interact and play a pivotal role in both
- 25 the humoral and cellular immune responses to T- cell dependent (TD antigens). In the absence of either molecule there is no isotype switching in response to a T cell dependent antigen, no germinal centre formation, and no enhanced secondary antibody responses (1,2)

- 30 The human CD40 receptor is a 48kDa, 277 amino acid polypeptide, transmembrane glycoprotein expressed predominantly at the B- cell surface. This receptor is also expressed by a number of other cell types. For example and not by way of limitation,

monocytes, basophils, eosinophils, endothelial cells, Langerhans cells, keratinocytes, Kaposi's sarcoma cells.

5 The human CD154 is a 33kDa, 261 amino acid polypeptide, transmembrane glycoprotein predominantly expressed at the T cell surface. This ligand is also expressed by a number of other cell types. For example, and not by way of limitation, mast cells, basophils, eosinophils, dendritic cells and monocytes. It is of note that CD154 has not been shown to be expressed in somatic cells other than those which are closely associated with the immune system.

10

Given the importance of the interaction of CD40 receptor with CD154 we have undertaken a study of the interaction between these molecules by expressing CD154 in cells which do not naturally express CD154, namely mouse fibroblasts expressing a mismatched MHC class, and used these cells as an immunogen. We anticipated that
15 this would act as a potent stimulator of the immune system.

It is known that blocking the interaction of CD40 with CD154 can suppress the immune response. For example, the use of anti- CD154 antibodies is known to abrogate the interaction between CD40 receptor and CD154 and result in attenuation
20 of the immune system in response to allografts (WO9856417 & WO9858669); suppression of autoimmune disease (WO9900143) and blood clotting disorders (WO9858672). We predicted that the recombinant expression of CD154 in MHC mismatched cells would promote an immune response to said cells. To our surprise, these cells did not promote an immune response but resulted in immune suppression
25 toward the injected transfected fibroblasts.

It is apparent that this observation has important implications with respect to allotypic recognition of implanted cells/tissues/organs. The expression of CD154 in cell types which do not naturally express this ligand resulted in failure of the immune
30 system to recognise the implanted cells as foreign. This has important implications with respect to tissue/organ transplantation and tissue/organ rejection by the host receiving the transplanted tissue/organ.

According to a first aspect of the invention there is provided at least one cell/tissue/organ for use in tissue engineering and/or organ transplantation wherein said cell/tissue/organ does not naturally express the CD154 ligand but is adapted to express at least an effective part of the CD154 ligand.

Reference herein to a part of CD154 includes reference to at least part of the extracellular domain.

10 In a preferred embodiment of the invention said cell/tissue/organ is transfected with DNA encoding at least the effective part of CD154, or a homologue thereof.

In yet a further preferred embodiment of the invention said DNA is genomic DNA.

15 In yet still a further preferred embodiment of the invention said DNA is cDNA.

In yet a still further preferred embodiment of the invention said cells/tissues/organ are of mammalian origin. Ideally said cells/tissues/organs are of human origin.

20 In yet still a further preferred embodiment of the invention said cells/tissues are selected from the following cell types: fibroblast; keratinocyte; osteoblast; chondrocyte; neurones, myocytes; hepatocytes; splenocytes, pancreatic β cells.

According to a second aspect of the invention there is provided a vector for use in the transfection of a selected cell/tissue/organ type for use in tissue engineering and/or organ transplantation characterised in that it contains a DNA molecule encoding at least an effective part of CD154 ligand, or a homologue thereof.

In a preferred embodiment of the invention said vector is adapted for the recombinant expression of CD154 ligand.

Conventionally, nucleic acid molecules used to transfect cells are referred to as vectors. Vectors used in genetic engineering are typically circular molecules, (although some may be linearised prior to transfection to facilitate the introduction of DNA into a host cell). Vectors of this type are referred to as plasmids, phages, or phagemids. In many examples these vectors have been genetically engineered to adapt them for expression in eukaryotic cells. For example, and not by way of limitation the provision of cell/tissue specific promoter elements which facilitate expression in a specific cell/tissue type; the provision of viral promoters which provide high levels of constitutive expression.

10

In addition to the above identified vectors, viral based vectors are used in transfection and in particular, gene therapy, to deliver genes to tissues *in vivo*. These vectors typically retain the capability to infect a host cell but are genetically modified to render the virus biologically disabled; this latter feature facilitates its removal from the organism and prevents its uncontrolled spread through host tissues. Examples of viral based vectors used in gene therapy include by example and not by way of limitation: adenovirus; retrovirus; parvovirus; and herpesvirus

15

In a further preferred embodiment of the invention said adaptation comprises the inclusion of appropriate expression control sequences which optimise the expression of the vector encoded nucleic acid molecule.

20

It will be apparent to one skilled in the art that said adaptation relates to a vector adapted for expression in a eukaryotic cell. For example, and not by way of limitation, said adaptation comprises the provision of constitutive, inducible, or repressible promoter elements; and/or the provision of polyadenylation control sequences for optimal expression; and/or the provision of selectable markers to allow the selection of said vector in a eukaryotic cell.

25

Furthermore the provision of regulatable promoter elements to control the amount of CD154 available to the cell/tissue is advantageous. It is desirable to regulate the amount of CD154 in accordance with the immune status of the host. For example,

30

once the cell/tissue/organ has been implanted it is may be beneficial to reduce the amount of CD154 expressed as the host becomes tolerant of the transplanted cell/tissue/organ. Alternatively, CD154 expression can be increased if the host begins to reject the donated cells/tissue/organ. This can be facilitated by modulation of the regulatable promoter to increase or decrease the amount of CD154.

In yet a further preferred embodiment of the invention there is provided a vector comprising a cell/tissue specific promoter sequence for use in the cell/tissue specific expression of CD154 according to any previous aspect or embodiment of the invention.

In a preferred embodiment of the invention said cell/tissue/organ is selected from the following tissue types: neuronal, muscle (e.g. smooth, striated, cardiac), bone, cartilage, liver, kidney, respiratory epithelium, endothelium, haematopoietic cells, spleen, pancreas, skin, stomach, intestine, oesophagus; blood vessels.

According to a third aspect of the invention there is provided a method to transfect a selected cell/tissue comprising:

- i. providing a vector according to the invention and adding said vector to cells/tissues;
- ii. incubating cells/tissues under conditions conducive to the introduction and maintenance of a vector;
- iii. exposing said cells to an agent at a concentration sufficient such that at least those cells/tissues including said vector are selected for; and optionally,
- iv. culturing said cells/tissues containing said nucleic acid molecule and, optionally, further still,
- v. storing said cell culture prior to use.

30

In a preferred method of the invention said cell/tissue is a mammalian cell/tissue. Ideally said mammalian cell/tissue is of human origin.

In a further preferred method of the invention, said transfection is transient. In the event that a transient transfection is required; steps (ii) and (iii) are not necessary.

- 5 It will be apparent to a man skilled in the art that where steps (ii) and (iii) are undertaken stable transfection is facilitated

Eukaryotic cells may be transfected via a variety of techniques. For example, and not by way of limitation, DNA may be introduced into mammalian cells via calcium
10 phosphate precipitation (Graham, FL and Van der Eb AJ, (1973) Virology 52, p456). This technique is particularly useful for both transient and stable transfection. An alternative to calcium phosphate precipitation is DEAE dextran mediated transfection (Gluzman, Y. (1981) Cell, 23, 175). This method is used primarily for transient transfection rather than stable transfection.

15 More recently, eukaryotic cells have been transfected using a pulse of high voltage electricity which, when passed through a culture of cells in the presence of vector DNA, momentarily results in permeabilisation of the cell membrane thus facilitating the introduction of vectors into said cells. This procedure is referred to as
20 electroporation. Furthermore, an alternative to the above mentioned methods is so called "ballistic" transfection where DNA coated microbeads are "shot" into cells/tissues to deposit the DNA into the cell/tissue.

According to a fourth aspect of the invention there is provided a therapeutic
25 composition comprising at least one cell/tissue according to any previous aspect or embodiment of the invention.

Preferably, said therapeutic composition is adapted for use in tissue engineering. More preferably still, said tissue engineering is the replacement of diseased or
30 damaged tissue.

Conditions which would benefit from therapeutic tissue engineering include by example, and not by way of limitation, arthritis and the replacement of joints; skin grafting for burn victims or injuries resulting in severe contusions; replacement of coronary arteries; replacement of diseased or damaged nerves and/or muscles; replacement of pancreatic β cells.

According to a fifth aspect of the invention there is provided at least one organ wherein said organ for use in organ transplantation comprises at least one cell/tissue according to any previous aspect or embodiment of the invention.

10

In a preferred embodiment of the invention said organ comprises at least one cell/tissue transfected with the vector according to any previous aspect or embodiment of the invention.

According to a sixth aspect of the invention there is provided a cell/tissue composition for use in cosmetic tissue engineering comprising at least one cell/tissue according to any previous aspect or embodiment of the invention.

In a preferred embodiment of the invention said cell/tissue comprises at least one cell/tissue transfected with the vector according to any previous aspect or embodiment of the invention.

20

According to an seventh aspect of the invention there is provided a method of treatment comprising;

25

- i) providing at least one cell/tissue which does not normally express CD154, or effective part thereof, and adapted so that same expresses at least the effective part of CD154;
- ii) administering said cells/tissues to a patient to be treated; and optionally,
- iii) monitoring the status of said cells/tissues by the patient.

30

According to an eighth aspect of the invention there is provided a method of treatment comprising;

- iv) providing at least one organ which does not normally express CD154, or
5 effective part thereof, comprising at least one cell/tissue expressing at least the effective part of CD154;
- v) surgical implantation of said organ to a patient to be treated; and optionally
- vi) monitoring the status of said cells/tissues by the patient.

- 10 When said cell/tissue/organ comprises an inducible promoter whereby CD154 may be selectively expressed said method may further involve monitoring/regulating the administration of at least one agent that activates said promoter.

- 15 In a preferred method of the invention said cell/tissue/organ comprises at least one cell transfected with a nucleic acid molecule encoding at least the effective part of CD154 according to any previous aspect or embodiment.

According to a further aspect of the invention there is provided a vehicle wherein said vehicle has least one cell according to the invention attached thereto.

20

Vehicle is defined as any structure to which cells according to the invention may attach and proliferate. For example and not by way of limitation, a prosthesis, implant, matrix, stent, gauze, bandage, plaster, biodegradable matrix and polymeric film.

25

In a preferred embodiment of the invention there is provided a therapeutic vehicle comprising cells according to the invention wherein said therapeutic vehicle is adapted to be applied and/or implanted into a patient requiring therapeutic tissue engineering.

30

In yet a further preferred embodiment of the invention there is provided a therapeutic vehicle comprising a matrix material (for example, and not by way of limitation, a

matrix material which is synthetic or naturally occurring and either long-lasting or biodegradable) comprising at least one cell according to the invention for use in surgical implantation procedures.

- 5 According to a yet further aspect of the invention there is provided a cosmetic vehicle comprising at least one cell according to the invention for use in cosmetic tissue engineering.

An embodiment of the invention will now be described, by example only, and with
10 reference to the following Figures;

Figure 1 is a representation of groups of five BALB/c mice immunised twice intraperitoneally with 5×10^4 L cells (closed circles), CD154 L cells (open squares), or PBS (closed triangles);

15

Figure 2 represents groups of five BALB/c mice immunised with L cells or CD154 L cells and boosted with either L cells or CD154 L cells;

Figure 3 represents groups of five BALB/c mice immunised with CD154 L cells or
20 PBS, immunised again with normal L cells 3 and 6 weeks later and bled 8 days after the last immunisation;and

Figure 4 represents measurements of serum nitrate levels of mice after immunisation with L cells or CD154 L cells.

25

MATERIALS AND METHODS

Cells and antibodies

30

L929 cells (L cells) and CD154 transfected L929 cells (CD154 L cells) were kindly provided by DNAX Research Institute, California. CD154 transfected L929 cells

were prepared as described elsewhere [15]. Anti-CD40 antibody 1C10 (9) was purified on a protein G column from hybridoma supernatant produced in a bioreactor by Sheffield hybridomas, Sheffield. The MR1 anti-CD154 mAb was purchased from Pharmingen.

5

Mice and Immunisations

BALB/c female mice of 8-12 weeks of age were obtained from the University of Sheffield Field Laboratories. L929 cells were removed from tissue culture flasks using EDTA (0.5mM), washed in PBS and 5×10^4 cells injected intraperitoneally into MHC mismatched BALB/c mice. Mice were bled 10 days post-primary immunisation and seven days after each subsequent immunisation.

10

Measurement of antibody responses

15

Antibody responses to L929 cell surface antigens were determined by Flow cytometric analysis. L929 cells, at 10^6 cells/ml, were incubated in FACS buffer (PBS, 3% BSA, 0.01% Sodium azide) at 4°C for 20 minutes with serial dilutions of mouse antisera for 20 minutes. Cells were then washed three times with FACS buffer and incubated with a FITC labelled goat anti-mouse immunoglobulins (Pharmingen) for 20 minutes at 1:100, washed three times and analysed using a Becton Dickinson FACScan analyser and "FacscanTM" and "LysisTM" software. Dead cells were gated out by forward and 90° angle light scatter. Mean fluorescence intensities (MFI) were plotted against dilution and examples of such plots are shown in figure 1. To simplify the remaining figures mean endpoint titres are shown, and were determined by the points of intersection of the MFI curves with those of normal mouse serum (i.e the PBS group from figure 1). Statistical analyses were by Student's t test.

20

25

Assay for Nitrate as a measure of Nitric oxide production

30

Sera were assayed for Nitrite levels (as an indicator of Nitric oxide production) 2h post injection with L cells or CD154 L cells by the addition of 50µl test serum to 100µl Greiss reagent (0.5% Sulfanilamide (w/v) 0.05 % Naphthylethylene (w/v) , 1.125% phosphoric acid). Sodium nitrite standards were double diluted across the plate starting at 100mM. The plates were incubated at RT for 10min and absorbance at 540nm measured.

RESULTS

Expression of CD154 on antigenic cells inhibits the alloantibody response

The antibody responses of BALB/c mice against normal or CD154 expressing L929 cells were not detectable by this flow cytometric assay following a single immunisation. However two i.p injections of L cells gave rise to antibody responses against L cells of around 1/1000 (Figure 1). In contrast mice immunised with CD154 L cells produced no detectable response against L cells even after two immunisations (Figure 1). We considered it possible that these apparent differences in immunogenicity were caused by antigenic variation between the two cell lines as responses were assayed against normal L cells; however similar results were seen when CD154 L cells were used as the antigen in the assay, Figure. Thus the results were due to a lack of response to the CD154 L cells in immunised mice.

25

CD154 L cells fail to prime for an alloantibody response against normal L cells, but do not induce tolerance.

30 Mice immunised first with CD154 L cells, and then with normal L cells failed to produce a normal secondary antibody response against the L cells, thus CD154 expression inhibited priming of the antibody response against L cells ($p=0.013$; Fig

2). It was possible that the CD154 L cells were inducing tolerance, leading to a lack of response on secondary exposure to normal L cells. To determine whether this was the case, two groups of mice were immunised with two doses of L cells, but one of the groups had previously been immunised with CD154 L cells. Had the CD154
5 expressing cells induced tolerance then the latter group should have produced a lower immune response. In fact this was not the case, and responses between the two groups were the same ($p=0.23$; Fig 3). Thus it would appear that CD154 expression does not result in tolerance, but rather a lack of recognition of the antigen on initial exposure.

10 **Induction of Nitric oxide by CD154 expressing L929 cells**

As CD40 ligation had been shown to enhance production of NO by macrophages [7] and NO production is responsible for a generalised immunosuppression seen in acute bacterial infection [8], we investigated whether NO levels were increased in mice
15 immunised with CD154 L cells. As shown in figure 4a, there was an increase in serum nitrate, indicating a rise in NO levels in vivo following immunisation with CD154 expressing cells which was greater than that seen after immunisation with normal L929 cells. It does not appear however that NO is responsible for the suppression of immune responses against CD154 L cells, as co-injection with the NO
20 synthase inhibitor, L-NAME abrogated the nitric oxide production but had no effect on suppression (Fig 4b)

The suppressive effect of CD154 expression is probably not related to the particulate form of the antigen

25

We have shown strong positive effects of CD40 ligation on immune responses to soluble antigens using anti-CD40 antibodies (6). However when the agonistic anti-mouse CD40 antibody 1C10 [9] (500 μ g) was co-injected with normal L929 cells, the antibody response was neither suppressed nor significantly enhanced ($p=0.33$; fig 4b).

30 The suppression mediated by CD154 expression is therefore unlikely to be related simply to the form of the antigen, administered as whole cells rather than soluble protein

Reversal of inhibition with anti-CD154

The inhibition of alloantibody responses by CD154 expression is clearly reversed by
5 pre-incubation of the cells with 10µg/ml MR1 (anti-CD154, 10) antibody ($p=0.0013$),
again indicating the suppression of responses by these cells is due to CD154
expression and not antigenic or other differences between these cells and normal L
cells (Figure 4b).

10 **DISCUSSION**

Interactions between CD154 and CD40 play a very important role in immune
responses. In general, stimulation of B cells through CD40 induces strong B cell
activation, proliferation and isotype switching especially in co-operation with
15 signalling by other factors such as antigen (or anti-IgM), and cytokines such as IL4.
Signalling through CD40 also appears to be important in initiating and maintaining
germinal centres [1]. Activation of B cells through anti-CD40 antibodies *in vivo* can
also give rise to enhanced isotype switching, greatly increased antibody responses
[6,11] and B cell proliferation [Dullforce, Greenwood and Heath, in preparation]. As
20 we had demonstrated strong adjuvant-like effects of CD40 ligation on antibody
responses *in vivo*, we considered that cell-surface expression of the CD40 ligand,
CD154, may be a potent means of enhancing anti-cellular immune responses. An
analogous approach had been used successfully utilising CD80 and CD86 transfection
to enhance the CTL response against tumour cells *in vivo* [12]. We therefore
25 examined the effect of transfection with CD154 on the alloantibody response to
murine L929 cells.

BALB/c mice (H-2^d) were immunised with the MHC mismatched cell line L929,
untransfected or stably transfected and expressing murine CD154 on the membrane.
30 Contrary to our expectations we found that expression of CD154, rather than
enhancing the alloantibody response to the L929 cells, actually suppressed the
response. This suppression only became evident after two immunisations because of

the poor primary response to normal L cells. However, the suppressive effect was mediated at the primary immunisation as the response to a second injection with normal L cells was suppressed by primary immunisation with CD154 L cells. The lack of responsiveness to CD154 L cells was not caused by antigenic differences
5 between the two cell lines, as similar results were obtained when CD154 L cells were used as antigen to detect the antibody. It appeared possible that the CD154 expression was rendering the L cells tolerogenic. However, that did not appear to be the case as mice immunised with CD154 L cells, followed by two doses of L cells, responded normally to the second dose of L cells. It appears, therefore, as though the expression
10 of CD154 by L cells simply prevents or suppresses effective priming or activation of the allospecific cells rather than induces tolerance.

Earlier experiments on the *in vivo* effects of CD40 ligation have been performed using soluble antigens and soluble anti-CD40 antibody []. It was therefore important
15 to determine whether the form of the antigen was the important factor in this apparent reversal of the effects of CD40 ligation. To this end mice were immunised with L cells and an agonistic anti-mouse CD40 antibody, 1C10 [] In this case the antibody response to the L cells was enhanced. Thus it would appear that the suppressive effect of CD154 expression on the L cells is dependent on: the extent of CD40 cross-
20 linking; the temporal differences between that cross linking by a cell surface antigen and that of soluble antibody; or qualitative differences between antibody and ligand induced CD40 signalling.

Of course immune responses to allogeneic cells are different in many ways to
25 immune responses against soluble antigens, not least in that the antigen itself is probably directly recognised by T cells without processing by a specialised APC. From the perspective of the B cell, encounter with a CD154 expressing L cell must be similar to an encounter with an activated T cell which may well also be expressing antigens which do not appear during B cell maturation, thus not allowing elimination
30 of B cells reactive with these antigens in the bone marrow. One of these would be the T cell receptor idiotype, and another may well be CD154. It has been proposed that autoimmune responses against newly expressed antigens (such as hormones and

breast milk) do not normally occur because of the lack of a "danger" signal. There is little evidence for CD154 expression in the bone marrow microenvironment, thus B cells expressing surface immunoglobulin specific for CD154 as well as TCR idiotopes might be expected to be present in the periphery. Because of the function of CD154, a CD154 specific B cell coming across it for the first time would receive a potent "danger" signal in the form of CD40 ligation. Thus it might be expected that strong autoantibody responses against CD154 would be produced. This is clearly not the case, and we propose that simultaneous and long-lived stimulation through surface immunoglobulin and CD40 and/or extensive cross-linking of the two receptors, may anergise the B cell preventing the production of large amounts of autoantibody against CD154 and possibly TCR idiotopes.

We consider that transfection of donor cells for transplantation with CD154 will have some role to play in enhancing the acceptance of allografts by the recipient.

REFERENCES

- 1) Kawabe, T., Naka, T., Yoshida, K., Tanaka, T., Fujiwara, H., Suematsu, S., Yoshida, N., Kishimoto, T and Kikutani, H. . Immunity 1, 167 1994
- 2) Xu J.C., Foy T.M., Laman J.D., Dunn J.J., Waldshmidt T.J., Elsemore J., Noelle R.J. and Flavell R.A. Immunity 1 423, 1994
- 3) Yang, Y and Wilson, J.M. Science 273, 1862, 1996
- 4) Van Essen, D., Kikutani, H and Gray, D. Nature 378, 620, 1995
- 5) Armitage, R.J., Fanslow, W.C., Strockbine, L., Sato, T.A., Clifford, K.N., Macduff, B.M., Anderson, D.M., Gimpel, S.D., Davis-Smith, T., Maliszewski, C.R., Clark, E.A., Smith, C.A., Grabstein, K.H., Cosman, D and Spriggs, M.K. Nature 357, 80, 1992
- 6) Dullforce, P., Sutton, D and Heath, A.W. Nature Medicine 4, 88, 1998

- 7) Tian, L.C., Noelle, R.J. and Lawrence, D.A. Eur. J. Immunol. 25:306, 1995
- 8) Alramadi, B.K., Meissler, J.J., Huang, D. and Eisenstein, T.K. Eur. J. Immunol. 22, 2249, 1992
- 5 9) Heath, A.W., Wu, W.W. and Howard, M. Eur. J. Immunol. 24, 1828, 1994
- 10 10) Noelle RJ, Roy M, Shepherd DM, Stamenkovic I, Ledbetter JA and Aruffo A. P.N.A.S (USA) 89, 6550, 1992
- 11 11) Ferlin, W.G., Severinson, E., Strom, L., Heath, A.W., Coffman, R.L. and Howard, M. Eur. J. Immunol. 26, 2911, 1996
- 12) Ramarathnam, L., Castle, M., Wu, Y. and Liu, J. J. Exp. Med. 179: 1205, 1994
- 15 13) Kaye, J and Janeway CA. J. Exp. Med. 159, 1397, 1984
- 14) Miyashita, T., McIlraith, M.J., Grammer, A.C., Miura, Y., Attrep, J.F., Shimaoka, Y and Lipsky, P.E. J. Immunol. 158, 4620, 1997
- 20 15) Randall, T.D., Heath, A.W., Santos-Argumedo, L., Howard, M.C., Weismann, I.L. and Lund, F.E. Immunity 8, 733, 1998
- 25